MODE OF OPERATION OF A SYSTEM OF CONTROLLING ELEMENTS IN MAIZE

Mode 6f Operation of a System of Controlling Elements
in Maize.

General descriptions of controlling elements in maize and of their

modes of operation have been given in a number of reports appearing in

recent years (for literature citations, see McClintock, 1956b).   It is

the purpose of t is report to consider some of the experimental methods

that have been employed to discover the mode of operation of the system

of controlling elements which has been referred to in other publications

as the Spm (Suppressor-mutator)-a_1^{m-1} system.   Controlling elements may be

defined as those unit components, carried in the chromosomes, that serve

to control gene action both with regard to type and degree and to the

tissues or parts of a tissue in which this will occur.   The different

controlling elements are recognized by means of their distinctive modes of

control of gene action, regardless of the primary type of action of the

gene substance itself.   They exhibit Mendelian inheritance patterns and

their locations in the chromosome complement may be determined by use of

ordinary genetic techniques.   However, they may undergo change in location

within the chromosome complement, appearing at new locations and disappearin

from previously determined locations, without losing their distinctive

properties in the process.   This process has been termed transposition and

the several methods that have been used to detect such transposition were

presented previously (McClintock, 1956b).

For many years, efforts of the author were concentrated on analysis

of the system composed of the two controlling elements designated Ac

(Activator) and Ds (Dissociation). This system was chosen for extended

studies because both elements ~~of this system~~ in it could be identified readily and

regardless of the location in the chromosome complement that each might

occupy. Thus, change in location of these elements, and the effects

produced when one of them is inserted at the locus of a known gene, could be

detected and subsequently analysed. Altogether, the operation of this

system at eight known gene loci has been examined. Also, two or more

independent insertions of one of these elements at four of these eight gene

loci were detected and the consequence of this examined in each case. It

was concluded from these studies that this system should be able to operate

at any gene locus provided that the effects of ~~its operation~~ this at a particular

~~gene~~ locus is not lethal. Most important, however, is the realization that

the mode of control of gene action may be predicted in advance, for it will

follow the rules that ~~characterize~~ apply to the operation of this system. Knowledge
discovered

of the mode of operation of the Ac-Ds system has been useful in guiding

experiments aimed at revealing the mode of operation of other control system

This applies particularly to methods for identifying controlling elements

and for detecting their transpositions. It has also provided a model for

recognizing and subsequently appraising the changes in state of the affected

gene locus. Such changes must be recognized if confusion in designing

experiments and interpreting results is to be avoided.

Some years ago, a large number of different variegates appeared in the

progeny of individual plants with a history of having been subjected to the

breakage-fusion-bridge cycle in their early development (McClintock, 1951).

At the time these variegates appeared, it was realized that it would not be

possible to examine each of them. Therefore, only a few among the many

that appeared were selected for subsequent study. The Ac-Ds system was

discovered among one of these selected cases; and as the mode of operation

of this system became apparent, attention was focused mainly upon it.
Study of
The other selected cases either wase discontinued or it was sharply curtailed

until adequate time could be found for more detailed examination. The system

responsible for control of gene action in one of these latter cases is now
the
sufficiently understood to allow conclusions to be drawn regarding/types of

controlling elements that are involved in it and their modes of operation.
operating
This is the system responsible for control of gene action at both a_1^{m-1}

and a_2^{m-1}, mentioned in previous reports. In this report, attention will

be given mainly to a_1^{m-1}.

Origin of a_1^{m-1}

The history of origin of a_1^{m-1} from a modification that occured at

a standard A_1 locus is as important for an appreciation of the controlling

elements involved in this system and their modes of operation as were the

histories of origin of modified gene action that appeared in the Ac-Ds

carrying cultures for an appreciation of the presence and mode of operation

of the elements of that system. The modified A_1 locus, designated a_1^{m-1},

was the third recognized case of change in gene action in a sequence which

commenced with an alteration at a previously unidentified gene locus concern

with chlorophyll production. The plants having this first member of the

sequence exhibited variegation for chlorophyll pigmentation. This variegat

was one of those ~~originally~~ selected among the many that appeared in the

original cultures, as described above. In the early stages of examination

of this variegate, a number of plants in one culture were self-pollinated.

On the ear produced by one of these plants, some kernels appeared that

exhibited variegation for anthocyanin pigmentation. Spots of deep

pigmentation appeared in a colorless background. The plants derived from

them also exhibited variegation for anthocyanin pigmentation. Subsequent

tests of these plants and their progeny indicated that an alteration had

occurred at the standard A_2 locus in one chromosome 5 of the parent plant.

This altered locus was given the designation a_2^{m-1}. Tests were then

undertaken to examine the changes in expression of gene action at a_2^{m-1} and t

determine the factors involved in control of this. In the course of
this study, a number of plants in a culture in which the system responsible
for control of gene action at a_2^{m-1} was present, were used as pistillate pare

in crosses with plants that were homozygous for the standard recessive, a_1,

in chromosome 3, and for the standard A_2 locus in chromosome 5. On one of

the ears this cross produced, a single kernel was found that exhibited

spots of anthocyanin/pigment in a non-pigmented background. A plant was grown

from this kernel and this plant, in turn, exhibited variegation for antho-

cyanin pigmentation. As ~~expected,~~ Tests crosses ~~utilizing both pollen and~~ with this plant

~~ears of this plant~~ indicated the presence in it of an ~~altered~~ modified A_1 locus, and

~~it~~ an altered locus was thereupon designated a_1^{m-1}. It was evident that ~~the alteration~~ some change

~~had occurred at~~ had occurred at the standard A_1 locus in one chromsome 3 in the ~~female~~ pistillate

parent plant, ~~and~~ This took place late in development of one of the ears ~~of~~ produced by this plant, for

only one kernel on this ear exhibited altered A_1 action. Studies aimed at

determining the components of the system responsible for control of gene

action at both a_1^{m-1} and a_2^{m-1} were contined but initially only on a

limited scale. Only recently has time been found to examine this more

completely. It is now known that the same system of control of gene action

operates at both a_1^{m-1} and a_2^{m-1}. Very likely this same system also

operated to control action of the gene that was associated with chlorophyll

production,--the initial variegate in the sequence. This postulate is

based ~~only~~ solely upon the patterns of variegation that were exhibited and upon

inheritance behavior for study of this case was discontinued some years ago

and therefore direct tests to determine identity of control systems cannot

now be made. Outline of the modes operation of the Spm-a^{m-1} Spt

In order to learn of the system that is responsible for control of gene

action at a_1^{m-1} and a_2^{m-1}, a large number of different types of tests were

conducted. The results obtained from each are consistent with one another

on the basis of the eventually determined modes of operation of the componen

of this system. In order to comply with space requirements, only a selecte

set of tests can be mentioned here. These are chosen in order to illustrate t

salient features of the mode of operation of this system and they will be

confined to studies conducted with a_1^{m-1}. Those conducted with a_2^{m-1}

will be given in a separate report.

From examination of the Ac-Ds system, it was learned that insertion

of the Ds element at the locus of a gene initiated the primary modification

that brought this action of the gene under the control of the Ds-Ac system. In many

cases, the action of the gene was noticably altered by this initial event and

detection of the presence of Ds at the gene locus could be made shortly after

it was inserted there. Subsequent modifications of the locus results from the effects Ac

exerts on the Ds element. The consequence of this is either removal of Ds

from the locus of the gene or a modification at the locus, induced by Ds,

that effects a change in its organization,--a change in state of the locus.

Both types of events can give rise to recognizable changes in action of the gene substance. With regard to a_1^{m-1}, insertion of a particular controlling element at the standard A_1 locus is considered to have occurred and to have been responsible for the initial change in gene action. Like Ds, it is this element that directly controls the type of gene action that will occur at the ~modified~ A_1 locus and ~also~ the types of change in this ~action~ that may occur subsequently. It appears to be the same element that is present at a_2^{m-1}. This conclusion is based on the response both of a_1^{m-1} and a_2^{m-1} to the presence of an independently located element designated Suppressor-mutator (Spm).

~When Spm is present, no antho or aim is produced in either~
~~All action of the gene substance at both a_1^{m-1} and a_2^{m-1}~~

~kernel or plant~ ~except in certain cells.~
~~is suppressed when Spm is present in the nuclei of a plant except in~~

~~certain cells~~ ~in these cells~ ~~where a change occurs to~~ the element residing at either a_1^{m-1} or a_2^{m-1} ~involves modifications~ that allows the gene substance to become active ~and this~ ~~either in~~

~activity may be observed~ ~cells.~
~~this cell or~~ in ~~the~~ descendant~~s~~. The particular type of expression of the gene that these modifications effect is thereafter maintained either in the presence or the absence of the Spm element. ~It may be concluded, then, that~ ~~In other words~~, the Spm element is complementary to the element located at a_1^{m-1} and a_2^{m-1}. It serves to activate it and as a consequence of this, stable mutations are produced. In this respect, it resembles Ac in the Ds-Ac system. However, /~the genic substance~ at both a_1^{m-1} and a_2^{m-1} may be active to some extent when Spm

is not present in the nucleus. When it is removed, either through meiotic

segregation or by means of somatic transposition, anthocyanin pigment may

appear in the kernels and plants having either a_1^{m-1} or a_2^{m-1} and its

distribution is uniform. In other words, there is no variegation. The

type and intensity of pigmentation is an expression of the particular state

of ~~either~~ the a_1^{m-1} or a_2^{m-1} locus that is present. These states and

their origins will be considered shortly. When Spm is returned to the

nuclei by appropriate crosses, ~~all~~ gene action at a_1^{m-1} and a_2^{m-1} is suppres

ed except in those cells where mutation-producing events occur. Thus,

Spm serves not only to activate the complementary element residing at

a_1^{m-1} and a_2^{m-1} and thereby conditions stable mutations at those two

loci, but it also must act upon this element in yet another manner for its

presence results in suppression of known potentials for gene action, except

in those cells where mutation-producing events occur. These two ~~seemingly~~

different aspects of the mode of control of the Spm element on the element

residing at a_1^{m-1} and a_2^{m-1} are responsible for its ~~being~~ designated dual action,

Suppressor-mutator. However, this seemingly dual action may be the

expression of only one process rather than of two unrelated processes, as wil

be indicated later.

The states of $a,^{m-1}$

All examinations of the effect of a controlling element at a gene locus

must be conducted with the affected locus. Since, in addition to stable

mutations, the controlling element **may** also initiate structural or organi-

zational modifications of the locus that alter its subsequent expression,--

that is, change the state of the locus,--it is necessary to consider

states of the affected locus and their origins before detailed results of

experiments are ~~can be~~ presented. With a_1^{m-1}, a changed state is readily

recognized by the appearance of an individual kernel or plant that

exhibits an altered response of the locus to the presence and absence of

Spm. ~~For example,~~ when the controlling element first entered the locus of

A_1, it effected a particular type of modification in the structure or

organization of the locus. In the absence of Spm, some gene action

occurred, at this locus. Kernels were lightly but uniformly pigmented as were the

plants derived from them. In the presence of Spm, however, all gene

action was suppressed except in some cells where mutation-producing

events occurred at thelocus of a_1^{m-1} that allowed the gene substance to

be active in the descendants of this cell. all ~~Each~~ such event did not

effect ~~result in~~ the same degree or type of gene action but many of them restored

the full or near full A_1 type activity. Many of these events occurred

relatively early in plant and kernel development. ~~Unlike Ac,~~ Differences ~~variations~~

in dose of Spm had no effect on altering the time of occurrence, their ~~of these~~

and in this respect the action of Spm differs from that of Ac.

events. ~~A number of~~ mutation-producing events ~~occurred~~ *also occurred* in germinal cells

and this made it possible to examine the nature of the mutation in the

next generation. Another type of change ~~at~~ a_1^{m-1} *was noted to have* also occurred in a

few of the germinal cells. These resulted in altered expressions of the

locus both in the presence and absence of Spm. Some of them were

detected initially in individual kernels in the progeny of the original

a_1^{m-1} carrying plant. When this plant was crossed to plants that were

homozygous for the standard recessive, a_1, which does not respond to Spm

but responds, instead to Dt, the ~~majority of kernels~~ ~~xxxxxxxan~~ a_1^{m-1}

~~unmodified ax xxxxxxxx~~ locus in the majority of kernels that received it

was unmodified. Among the kernels receiving the unmodified a_1^{m-1} locus and

also Spm, the variegation pattern was much the same. There were many

large ~~patches of unexamined tissues~~ *pigmented areas,* indicating early occurring mutation-producing

as well as some smaller pigmented areas.

events. Many of these exhibited the full A_1 type of pigmentation.
areas

However, an occasional kernel appeared that exhibited a quite different

pattern of pigmented areas. (Two kernels) were found, among several

thousand that had only small pigmented areas. These *areas* were uniformly

over the aleurone layer

distributed ~~and~~ the intensity of pigment in ~~them~~ *the areas in one of these kernels* was either quite light

in the other kernels all of the pigmented areas were dark, but the pigment filled

or very dark. Another kernel appeared that had a number of large

pigmented areas as well as some small areas but the intensity of the

pigment in all of them was ~~much less than that given by the standard A~~ relatively light. A_1.

It ranged from very faint in some areas to ~~a medium intensity~~ *dark* pale in others.

Plants were ~~grown~~ grown from the three described/ exceptional kernels. Tests conducted

with them and their progeny indicated that the pattern of variegation

exhibited in the presence of Spm was heritable.   The altered a_1^{m-1} locus,--

the changed state of the locus,--in each case responded in its own

particular way to the presence of Spm and also to its absence.   In the

absence of Spm, the state of a_1^{m-1} ~~present~~ in the first described kernel

produces ~~deeply pigment~~ deeply pigmented kernels and plants.   That in the

second described kernel gives rise to very lightly pigmented kernels but

rather darkly pigmented plants.   The state of a_1^{m-1} present in the third

described kernel produces no pigment in either plant or kernel in the

absence of Spm.   Subsequently, other states of a_1^{m-1} ~~have been~~ were isolated.

Each is distinguishable from the other by the types of mutation and the

time and frequency of their occurrence ~~in the presence of Spm~~ that appear when Spm is present as well as

the type of expression of the gene substance that ~~occurs~~ appears in the absence of

In all cases, gene action is suppressed in the presence of Spm except in those cells (and their descendants) in which mutations

Spm.   No relationship was noted among the different states between the

control of time and frequency of occurrence of mutation in the presence of Spm and that

of type of gene action that occurs in its absence.   Figure 1 illustrates

the distinctiveness of several of the states of a_1^{m-1}, and table 1 records the range of differences that is expressed among the presently isolated states of a_1^{m-1}.

The integrity of a state of a_1^{m-1} is maintained in heterozygous

plants and this applies to plants that carry a different state in each of

their chromosomes 3.   In such plants, each state responds to Spm in its

own predictable way and the variegation patterns each produces will be

expressed in the plant or kernel tissues.   Figure 2 illustrates this.   The

kernels in the photographs carry the state shown in — of figure 1 in one

chromosome 3 and the state shown in — of figure 1 in the homologue.   The

pattern of mutation produced by each state ~~as~~ and the mutation type readily recognized in these

kernels.   In the plants, normal segregation of the two states occurs at

meiosis and each may be recovered in the expected proportion from the

gametes that these plants produce.

States of a_1^{m-1} are maintained unchanged in the absence of Spm.   In

its presence, however, new states may arise and the frequency of occurrence

of this in germinal cells is related to the time of occurrence of mutation-

producing events that a particular state exhibits.   If the state produces

some early occurring mutations, then new states of a_1^{m-1} may appear in the

germ cells, and the frequency of this is proportional to the frequency of

occurrence of these early mutations.   If, on the other hand, the state is

one in which mutations occur only late in development of the plant, or

~~kernel~~, then few or no altered states may be recovered in the gametes of

these plants.   The mutation-producing events at a_1^{m-1}, regardless of state, give rise to stability of expression of the locus.   The particular type of gene action the mutational event produces continues to be expressed in subsequent generations both in the presence and in the absence of Spm. This suggests that the change responsible for these mutations may ~~have~~ removed the controlling element from the locus or it may ~~have~~ result~~ed~~ in its inactivation with regard to Spm.   Since the changes in state arise at the very same developmental period as the mutation-inducing events occur, it is concluded that they represent a modification at the locus induced by the controlling element residing there that did not result either in its removal or $\overset{\underset{\wedge}{in\ its}}{}$ inactivation.   In other words, the element responded to the presence of Spm at the appointed time and in the appointed cells but the consequence of this was not the usual one,--i/e/, the mutation-producing event.   Instead, the responding element itself was either modified or it induced some reorganization at the locus that modified its capacity to respond to Spm with regard to time ~~when this will occur~~ $+$ frequency of occurrence of mutations, as well as, the types of mutations it can induce, ~~as well as~~ $and\ also\ with\ regard\ to$ the type of gene action that can occur in the absence of Spm.   It is as evident in this case as it is with Ds that a change in state is one of the consequences of the response of the

of the controlling element residing at a gene locus to the independently

located element of the system,--Spm in this case and Ac in the case of Ds.

Detection of the Spm element and its mode of operation
~~Transpositions of Spm~~

In the early examination of a_1^{m-1}, no evidence of the presence of the

Spm element, ~~was detected~~. ᵀhis was because the original a_1^{m-1} carrying

plant had a number of Spm elements located at various positions in the

chromosome complement. All or certainy nearly all of the gametes it produced had Spm elements

~~In~~ them. Since the dose of Spm has no appreciable effect on the pattern

of mutation produced by a_1^{m-1}, differences in number of Spm elements in a

kernel or plant is not made directly evident by this means as it is with Ac.

It was only after several generations of crossing of a_1^{m-1} carrying plants to a_1

tester stocks which did not have Spm, that clearly expressed mendelian type ~~definite~~ ratios of variegated to

uniformly pale
~~light~~ colored kernels appeared on the test cross ears. These ratios

indicated the presence in the a_1^{m-1} carrying plants of an independently

located element that is associated with control of a_1^{m-1} expression.

They also indicated that the number and the location of this element was not

the same in all tested plants. In the meantime, several different states

of a_1^{m-1} had been isolated on the basis of the altered variegation patterns

that appeared in individual kernels on several of the ~~first~~ testcross

ears, as described earlier. In successive generations of crossing of

plants carrying these different states of a_1^{m-1} to the a_1 tester stocks,

the same kinds of ratios of variegated to non-variegated kernels also

began to appear.   It was then assumed that the uniformly pigmented

(non-variegated) kernels and plants carried a_1^{m-1} but not the independently

located element.   On the other hand, this element was assumed to be

present in those kernels and plants that showed pigmented areas in a non-

pigmented background.   On this interpretation, the independently located

element was exerting a suppressor-mutator type of control of gene action

at a_1^{m-1}.   Since the dose of this element obviously did not affect the

pattern of mutational events, control of this must reside at the a_1^{m-1}

locus itself, the type depending upon the state of the locus.   It was

evident that the phenotypes of the nonvariegated kernels and plants also

reflected the state of the a_1^{m-1} locus that was present in them.

The hypothesis stated above was subject to test.   If it were correct,

evidence in support of the following four statements should be obtained:

(1) All variegated kernels and plants carry at least one Spm element.

(2) No Spm element is present in the non-variegated class of kernels

and plants.(Germinal mutations, mentioned ~~described~~ earlier, are excluded from this

class.)

(3) The a_1^{m-1} locus in the non-variegated kernels and plants is capable

of responding to Spm if this element is subsequently introduced into a

nucleus.

(4) The type of response to Spm and the phenotypes produced in its

absence is a function of the state of the a_1^{m-1} locus.

A large body of evidence in support of these statements is now available

and it has been obtained by various types of test, only a few of which

need be outlined here.

In order to facilitate identification of the presence or absence of

Spm in a particular plant, so-called Spm tester stocks were developed.

These stock have either one or the other of the two states of a_1^{m-1} shown

in ─ and ─ of figure 1, and these states were selected for the following

reasons. In the first place, when Spm is present, very few germinal

mutations or changes in state occur. Therefore, nearly all of the

gametes produced by plants carrying these states of a_1^{m-1} and also Spm

have an unmodified a_1^{m-1} locus in them. Secondly, the pattern of variega-

tion each produces in the presence of Spm is distinctive and non-obscuring.

Thirdly, in the absence of Spm, one of these states,─ figure 1, gives rise

to darkly pigmented kernels and this is a useful character in some tests.

The tester stocks were made homozygous for one or the other of these two

states. They were also made homozygous for either Sh_2 or sh_2, located

very close of a_1^{m-1} (about one-quarter of a percent crossing over occurs between them).

An example of one series of tests will illustrate some of the methods employed to determine Spm constitutions of individual plants. The silks of two ears of a variegated plant carrying a_1^{m-1} and Sh_2 in one chromosome 3 and a_1 and sh_2 in the homologue and also Y in one chromosome 6 and y in its homologue, recieved pollen from a plant that was homozygous for a_1, sh_2 and y. The state of a_1^{m-1} in the pistillate plant was that shown in - of figure 1. From this cross, the two ears produced a total of 745 kernels. There were 181 Sh_2 kernels in which the aleurone layer was uniformly and rather darkly pigmented; 69 of these were Y and 112 were y. The aleurone layer in another 188 Sh_2 kernels exhibited a number of spots of the full A_1 type pigment in a colorless background and 117 of these were Y and 71 were y. The aleurone layer in the remaining Sh_2 kernels was completely colorles and the starch in its endosperm was y. Among the 375 sh_2 kernels on these two ears, the aleurone layer in 373 of them was totally colorless; 186 of these kernels were Y and 187 were y. The remaining 2 sh_2 kernels exhibited spots of full A_1 type pigment in a colorless background. The phenotype of the starch in one of them was Y and that in the other was y. Since

a_1^{m-1} is colsely linked to Sh_2, nearly all of the Sh_2 class of kernels on

these two ears should carry an a_1^{m-1} locus and nearly all of the sh_2

kernels should be homozygous for the standard recessive, a_1.   The close

linkage of a_1^{m-1} to Sh_2 is obvious for only 1 Sh_2 kernel in the total of

370 was completely colorless and only 2 sh_2 kernels in a total of 375 had

pigment in the aleurone layer.

On the basis of the interpretation given above, the uniformly

pigmented kernels should have no Spm in them whereas those exhibiting spots

of the full A_2 type pigment in a colorless background should have this

element.   From the ratio of these two classes among the Sh_2 kernels

(181 to 187) it could be concluded that the variegated pistillate parent

plant had one Spm.   The ratio of Y to y in each of these two classes

indicated that this Spm element was carried in the Y bearing chromosome

It was then necessary to determine whether or not these conclusions were

valid.   For this purpose, 104 plants were grown from various types of

kernels on these two ears and tests were conducted with them.   The

phenotypes of the selected kernels were as follows: 11 uniformly pigmented

Sh_2 Y, 13 uniformly pigmented. Sh_2 y; 17 variegated Sh_2 Y, 8 variegated

Sh_2 y, 1 variegated sh_2 Y, 30 colorless sh_2 Y, and 24 colorless sh_2 y.

All 24 plants derived from the uniformly pigmented kernels were themsleves

uniformly pigmented. All ~~of the~~ 26 plants derived from the variegated

kernels showed small streaks of the A_1 type pigment in a non-pigmented

background. And, as expected, all 54 plants derived from the colorless

sh_2 class of kernels lacked anthocyanin pigment. Each plant was then

tested for presence or absence of Spm by crossing it with a plant in an

Spm tester stock.

To illustrate how the tester stocks can serve to reveal the presence

or absence of Spm, those tests conducted with the 54 plants derived from

the colorless, sh_2 class of kernels will be considered first. This is a

completely objective test since the presence or absence in any one of them

of Spm could not be assumed on the basis of phenotypic expressions. / The silks of One

or more ears of each of these plants received pollen from plants that were

homozygous for either state - or - of figure 1, for Sh_2 and for y; these

pollen parents were uniformly pigmented indicating the absence of Spm in

them according to the stated hypothesis. If the plant being tested has no

Spm, then all of the kernels on the resulting ear will be uniformly colored.

If, however, the plant being tested carries Spm, then it should be present

in some of its gametes. Following introduction of the a_1^{m-1} locus from

the male parent, the presence of Spm in those kernels that received it from

the female parent should be revealed by the appearance in them of small,

The control Spm events?

deeply pigmented spots in a colorless background due to ~~activation of the~~

~~the~~ a_1^{m-1} locus ~~by the Spm element~~.   In those kernels that did not receive

Spm, the aleurone layer should be uniformly pigmented.   Among the

30 plants derived from the colorless, sh_2, Y class of kernels, it could

be determined on this basis that 15 had a single Spm element and 15 had no

Spm.   In 13 of the 15 plants that had Spm, linkage of it with Y was

evident (A, table 2) but in the 2 remaining plants, no linkage of Spm with

Y was evident (B, table 2).   (The reason for the absence of/Spm with the Y
linkage of

in these 2 plants will be considered ~~in~~ ~~the next section~~ *later*.   It need only be

mentioned here that this is not unexpected.)   Among the 24 plants derived

from the colorless, sh_2, y class of kernels, 6 had a single Spm element

(C, table 2),and 18 had no Spm.

Each of the 24 plants derived from the uniformly pigmented kernels

~~was uniformly pigmented.   Each~~ was tested for presence or absence of Spm

in the described manner and in none of them was Spm found to be present.

All of the plants derived from the variegated kernels showed streaks of the

A_1 type pigment in a non-pigmented background and the test crosses indicated

the presence of Spm in each of them.   In 16 of the 17 plants derived

from the variegated Sh_2 Y class of kernels, one Spm element was present and

in 15 of these plants, it was linked with Y (D, table 2).   In one ~~of these~~

plant, however, no evidence of linkage of the single Spm element with Y was noted (E, table 2). In the remaining plant in this group, 2 Spm elements were present, neither of which was linked with Y (F, table 2).

More than one test cross ear was obtained from 11 of these 17 plants and the number and location of the Spm element was the same in the cells producing all ears except for one plant. In this plant, one Spm element was present in the cells producing the main ear and it was linked with Y (G-1, table 2). In the cells that produced the tiller ear, however, a single Spm element was present but it showed no linkage with Y (G-2, table 2).

In all 8 plants derived from the variegated Sh_2 y class of kernels, one Spm was present (H, table 2). One Spm element was also present in the plant derived from the variegated sh_2 Y kernel. This plant was used as a pollen parent in crosses with plants having different constitutions: [1] homozygous also for
for a_1 and sh_2/ and $\bar{y}$ and having no Spm, [2] homozygous for several different states of a_1^{m-1} but having no Spm, and [3] to plants heterozygous for different states of a_1^{m-1} that were a_1^{m-1} Sh_2/a_1 sh_2, y/y, no Spm.   In this third group, plants with different states of a_1^{m-1} were represented.   All tests indicate the presence in the pollen parent of a_1^{m-1} in one the sh_2 carrying chromosome and of an element Spm in the Y carrying chromosome, and that this Spm element was capable of activating the various different states of a_1^{m-1}.

The tests described above were conducted with the progeny of a single plant in a culture. There were 19 variegated plants in this culture. Each was derived from a variegated kernel that appeared on an ear of a variegated plant that was a_1^{m-1} $Sh_2/$ a_1 Sh_2, Y/y, wx/wx (chromosome 9), pr/pr (chromosome 5) in constitution when pollen of a plant homozygous for a_1, sh_2, y, Pr, and Wx and having no Spm had been placed on the silks of this ear. All kernels appearing on t is ear were Sh_2 and the distribution of phenotypes among them were as follows: 28 uniformly dark pale Y, 7 uniformly dark pale y, 55 variegated (spots of deep pigmentation in a colorless background) Y, 86 variegated y, 75 colorless Y, and 103 colorless y. Among the kernels showing anthocyanin pigment, the ratio of uniformly dark pale colored kernels to variegated kernels indicated the presence of at least 2 and possibly 3 Spm elements in the pistillate parent plant and one of these appeared to be linked with Y. The silks of ears of 9 plants derived from the variegated Y class of kernels on t is ear and of 10 plants derived from the variegated y class received pollen from plants that were homozygous for a_1, sh_2, and y, and had no Spm. The ratio of kernel types appearing on the resulting ears produced by each of these 9 plants is entered in table 3. In this table, the 9 plants are placed in four groups, A to D, according to the assumed constition of Spm in each that the ratio of

kernel types suggested.   The 6 plants in A of this table were assumed to

have a single Spm element located in the Y bearing chromosome.   Progeny

from 4 of these 6 plants were grown and again tested for Spm.   Those

derived from plant 6629A-1, line 1 of A of table 3, were considered separate

above.   The total number of progeny or plants in this A group that were tested

and their phenotype of the kernel from which they arose origins are entered in the last line of A of table 3.   These

tests verified the presence of Spm in the variegated kernels and plants and

Its absence in the uniformly pigmented kernels and plants.   They also

verified the assumed Spm constitution and location in the pistillate

parent plant.   However Only those tests conducted with the 116 plants derived

from the colorless sh_2 class of kernels will be summarized here.   Among

the 56 plants derived from the a_1 sh_2 Y class of kernels, 32 carried Spm

and 24 had no Spm.   In 30 of the 32 plants having Spm, linkage of it with

Y was expressed, I, table 2.   In two plants, the Spm element did not appear

to be linked with Y, plants were mentioned earlier (B, table 3).   Among the 60 plants

derived from the a_1 sh_2 y class of kernels, 17 had a single Spm element

(J, table 2) and 43 had no Spm.

In order to verify the absence of linkage of Spm with Y location in the 2

plants entered in B of table 2, tests of some of the progeny of both of them

were conducted.   An ear of one of these plants had been self-pollinated

and another ear of this plant had been used in the cross with an a_1^{m-1} Sh_2 y, no Spm tester plant. The Progeny from both of these ears were again tested for Spm constitution and location. The silks of ears of 8 plants derived from a_1 sh_2 kernels in the Y class on the self-pollinated ear received pollen from the a_1^{m-1}, Sh_2 y, no Spm tester plants. From the kernel types on the resulting ears it could be concluded that 2 of these 8 plants were Y/Y in constitution and that one of them had no Spm whereas the other had 1 Spm (199 pale colored kernels : 169 variegated kernels on the test cross ear). The remaining 6 plants were Y/y. One Spm was present in 4 of them but it was not linked with Y (K, table 2). An Spm element appeared to be carried at allelic positions in a pair of homologous chromosomes in the remaining 2 plants (L, table 2). Seventeen plants derived from the variegated Sh_2 Y class of kernels the on testcross ear of this same parent (column 6, K, table 2) plant were crossed by plants in the Spm tester stocks. In 16 of these 17 plants, one Spm was present and on none of the ears produced by 15 of them was their any evidence of linkage of Spm with Y (M, table 2). However, the ratio of kernel types appearing on the test cross ear of one of them suggested such linkage (N, table 2). The remaining plant had 2 Spm elements, neither of which was linked with Y (O, table 2).

Tests of Spm constitution in 9 plants derived from the variegated Sh_2

Y kernels on the ear produced by the test cross with the other plant

entered in B of table 2, suggested that the Spm element in this plant had

been carried in the Y bearing chromosome but at a new location that was

farther removed fr m Y.   Seven of the 9 progeny plants had a single Spm

element and in all of the test cross ears, loose linkage of ~~it~~ Spm with Y was
<small>they produced</small>

expressed (P, table 2).   One plant of the 9 had 2 Spm elements (Q, table 2)

and ~~in~~ the remaining plant had 3 Spm elements (R, table 2).

Further examples of the progeny test method of determining Spm

constitution and location will not be given here.   It should be mentioned,

however, that such tests were conducted with the indicated progeny of

the plants entered in B, C, and D of table 3, and also with the progeny of

the 10 other plants of this same culture.   Also, ~~a number of~~ such tests
<small>many additional</small>

were conducted with the progeny of plants having other states of a_1^{m-1}
<small>and none of them were</small>

or combinations of states, i.e., carrying different states of a_1^{m-1} in

ech chromosome 3.   ~~The~~ tests clearly support the 4 statements given on
<small>All these</small>

page   , and particularly statements (1) and (2).   More direct support for

statements (3) and (4) was obtained by other tests to be described shortly.

Various different locations of Spm, either in different chromosomes of the

complement or in different locations within the same chromosome, were

discovered in these tests and each determined position of it was

subsequently verified by means of progeny tests. Howev r, it is clear

from the tests so far described that Spm does not remain at one location

within the chromosome complement but disappears from a known location and

appears at a new location and this will be considered in a separate

section. Before this is ~~described~~ discussed, it is necessary to show that the

Spm element, regardless of location, is capable of acting upon any one of

the states of a_1^{m-1}.

Responses of different states of a_1^{m-1} to the same Spm element

Evidence for statement~~s~~ (3),+(4) page _____, appeared in all tests in which

either one of the two states of a_1^{m-1}, present in the tester stocks, had

been used in crosses with plants carrying Spm. Each state responded to

Spm in its characteristic manner. In order to determine whether the

Spm element present in a particular plant would be capable of activating

other states of a_1^{m-1}, several additional types of test were performed.

One of them utilized different ears produced by a single plant. In one

such test, ~~these~~ were selected that plants were homozygous for a_1. Spm could be either

present or absence in any one of them. Pollen from a plant of ~~the~~ a

tester stock carrying either state — or state — was placed on the silks of

an ear of one such plant. The silks of ~~another~~ a second ear of the same plant

received pollen from ~~another~~ a plant that was homozygous for a different

state of a_1^{m-1} and in which no Spm was present. If the pistillate plant

had no Spm, then all kernels on both ears were uniformly pigmented, and the

intensity of this reflected the state of a_1^{m-1} introduced by the pollen

parent (excluding state —, figure 1, which gives colorless kernels in the

absence of Spm). If, however, the pistillate parent carried Spm, then

both variegated and non-variegated kernels appeared on ~~both~~ each ears but the

phenotypes of the two classes of kernels on each ear, a third reflected the state of

a_1^{m-1} introduced by the pollen parent.   Again, if the ~~activating~~ Spm element

✓showed linkage with a genetic factor ~~among the kernel~~s on one ear, the

~~usually showed this~~ was usually expected same linkage on the other ear, and an example of this

is given in S of table 2.   In this test, the main ear of an a_1/a_1, Y/y

plant received pollen from one ~~the~~ of the Spm tester stocks.   The kernel

types on the ear this cross produced, S-1, table 2, indicated the presence

~~in~~ the pistillate parent of an Spm element carried in the Y chromosome.

The silks of a tiller ear of this same plant received pollen from a plant

that was homozygous for state $\stackrel{\text{(sheer)}}{-}$, figure 1, and also for y.   The pollen

parent was uniformly pigmented indicating the absence of Spm in it.   The

kernel types on the ear this cross produced are entered in S-2 of table 2.

The ratio of variegated to non-variegated kernels in the Y and y classes

was much the same on both ears.   Thus, it could be concluded that the Spm

element, carried in the Y chromosome of the pistillate plant, was capable

of activating either state of a_1^{m-1}.   Pollen from the same collection

that was used in the latter cross was also placed on the silks of a plant

homozygous for a_1 and for y, but in which Spm was known to be absent.   All

of the 294 kernels on the ear this cross produced were uniformly lightly

pigmented and all were y.   This test confirmed the absence of Spm in the

pollen parent.

Another type of test that was employed to indicate the response of

different states of a_1^{m-1} to the same Spm element, utilized the pollen of

plants that were homozygous for a_1 and in which $\cancel{5}^{one}$ Spm element was present.

The types of test conducted with two such plants, number 6861-1 and 6861-7,

are illustrated in table 4 .   Both of these plants were homozygous for

a_1 and sh_2, ~~and a single Spm element was present in each as shown by the~~

~~ratios appearing in the table.~~ ~~Rathxxianix~~   The silks of an ear of each

plant received pollen from the a_1^{m-1} tester stock that carries state 5718.

that appeared

The types of kernels on the resulting ear are entered in A of table 4 .

of these two

These ratios indicate the presence of $\cancel{5}^{one}$ Spm in each plant.   Both plants

were used as pollen parents in crosses with plants that were homozygous for

state 5719A-1 but in which no Spm was present.   The types of kernels

on the ears resulting from these crosses are entered in B of table 4 .

Again, a 1 : 1 ratio of variegated (Spm) to non-variegated (no Spm) kernels

appeared on these ears, indicating the presence of 1 Spm in each of the two

pollen parents.   These same two plants were also used as pollen parents in

crosses with plants that were a_1^{m-1} $Sh_2/$ a_1 sh_2 in constitution and had no

latter

Spm.   The state of a_1^{m-1} in these plants was that which gives no anthocyani

pigmentation in the kernel and plant in the absence of Spm (state 5720, -

produces

figure 1) but ~~gives~~ many mutations to the lower alleles of A_1 in its presenc

Other ears of these same plants received pollen from plants that were

homozygous for a_1 and sh_2 but had no Spm. The types of kernels appearing

on the ears resulting from each of these two types of cross are entered in

C of table 4. Again, it is evident that plants 6861-1 and -7 each have

one Spm element that is capable of acting on this state of a_1^{m-1}. The

results obtained from the described tests are those to be expected if the

Spm element in plants 6861-1 and -7 is capable of acting on different

states of a_1^{m-1}. The same type of test as that just described was

conducted with $a_1\ sh_2/a_1\ sh_2$ plants having more than one Spm element and

the ratio of kernel types on the test cross ears was that expected if each

of the Spm elements present in the $a_1\ sh_2/a_1\ sh_2$ plant was capable of

acting on each of the states of a_1^{m-1}.

Still other types of test were conducted to determine the capacity of a

Spm element to act on different states of a_1^{m-1}. One of them utilized the

pollen of a plant that was $a_1^{m-1}\ sh_2/a_1\ sh_2$ in which a single Spm element

was present at a known location in the chromosome complement. When such a

plant was used as a pollen parent in crosses to plants that were $a_1^{m-1}\ Sh_2/$

$a_1\ sh_2$ and having no Spm but among which different states of a_1^{m-1} were

represented, the types of kernels on the resulting ears clearly indicated the

capacity of the Spm element in the male parent to act not only upon the state

of the a_1^{m-1} locus delivered by the male parent, but also upon the state of

the a_1^{m-1} locus delivered by the female parent. Again, when plants

carrying Spm that were a_1^{m-1} Sh_2/ a_1 sh_2, among which different states of

a_1^{m-1} were represented, were used as pistillate parents in crosses with a

plant that was homozygous for one state of a_1^{m-1} and also for sh_2 but in

which no Spm was present, activation of the a_1^{m-1} state delivered by the

male parent by the Spm element delivered by the female parent was indicated

in all tests of this type.

Detailed consideration of the various types of test mentioned above

cannot be given here. However, all of them clearly established the

similarity of the Spm element carried in the many different tested plants,

and regardless of its number or its location in the chromosome complement

of a given plant. They also established the ability of the Spm element

to act upon any of the selected states of a_1^{m-1} and they indicated that

control of type of gene action in the absence of Spm and control of the

time and frequency of occurrence of mutation, as well as its type in the presence of Spm is solely a function of the

state of a_1^{m-1}.